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Cathepsin B and its interacting proteins, bikunin and TSRC1, correlate with TNF-induced apoptosis of ovarian cancer cells OV-90

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Abstract **Increasing evidence suggests that lysosomal cysteine proteases cathepsins contribute to the progression of cell apoptosis. Here we found that apoptosis of ovarian cancer cells OV-90 triggered by TNF was cathepsin B-depended. Two cathepsin B binding proteins, bikunin and TSRC1, were identified by yeast two-hybrid method and the interactions were confirmed in vitro and in vivo. Overexpression of bikunin could suppress TNF-induced apoptosis of OV-90 cells, and TSRC1 overexpression had an opposite effect on apoptosis. The presented results suggest that cathepsin B and its interacting proteins, bikunin and TSRC1, are involved in the apoptotic pathway of ovarian cancer cells.**

Keywords: Cathepsin B; TNF; OV-90 cells; Apoptosis; Bikunin; TSRC1 protein

1. Introduction

Apoptosis is an active form of cell death by which multicellular organisms eliminate damaged or otherwise unwanted cells. The apoptosis process is controlled by a number of complex proteins which are activated by various triggers and arranged in sequential signaling modules. Apoptosis can be initiated either through death receptors on the cell surface, an extrinsic pathway or through mitochondria, an intrinsic pathway. Both pathways converge to a final common pathway involved in the activation of a cascade of caspases. The activated executioner caspases cleave key regulatory and structural molecules, culminating in cell death [1].

Tumor necrosis factor (TNF) is a pleiotropic cytokine capable of eliciting complex and diverse cellular events, including apoptosis, cell growth and proinflammatory genes expression [1,2]. TNF triggers cell apoptosis by binding to TNF receptor1 (TNFR1). Binding of TNF leads to the trimerization of TNFR1 and subsequent recruitment of TNFR1-associated death domain protein (TRADD), TNF receptor associated factor 2 (TRAF2), and the death domain kinase RIP. These proteins form a plasma membrane bound complex (complex 1). After dissociation from TNFR1, complex 1 components TRADD, TRAF2 and RIP associate with Fas associated death domain protein (FADD) and caspase-8, forming a cytoplasmic complex (complex 2). And then caspase-8 is activated in complex 2, followed by the activation of downstream executioner caspases and final cell death.

TNF can concomitantly induce the gene expression that promote cell survival through the TNF receptor complex associating proteins TRAF2 and RIP1, which activate NF-κB and MAPKs signaling cascades. The specific consequences of TNF signaling depend on the cell type and context. Some cells can be susceptible to TNF cytotoxicity by selective blockade of the NF-κB pathway or by general inhibition of transcription or translation [2,3].

It is a general goal to seek factors to control apoptosis and to understand functions of the factors in the apoptosis. Based on existing data, caspases are well recognized as a key player and other proteases such as cathepsins, calpains, and serine proteases may account for alternative types of apoptosis [4]. Cathepsins, a family of cysteine proteases, are normally localized in lysosomes serving as simple housekeeper enzymes involved in intracellular protein catabolism [5]. Recently, it is found that the leakage of lysosomal cathepsins into cytosol caused by some agents or stimuli can result in apoptosis of some cells [6–8]. For example, p53 in M1 mouse myeloid leukemic cells induces apoptosis and this type of apoptosis is initiated by lysosomal destabilization [7]. Moreover, some microtubule stabilizing agents such as paclitaxel induce cell death in non-small cell lung cancer cells, and this type of cell death is not depended on caspases while caused by disruption of lysosomes and release and activation of cathepsin B [8]. Cathepsin B is the most abundant cysteine protease in lysosomes, and based on recent reports, it is involved in apoptosis [6,9–11]. The typical studies in the cathepsin B-knockout mice have recognized the cathepsin B as a potent inducer of apoptosis [9]. At the same time, there exist different reports regarding mechanisms for role of cathepsin B in the apoptosis. When some cells are treated by TNF, cathepsin B released from ruptured lysosomes into cytosol activates caspase-dependent or caspase-independent pathways of cell death [10–12].

The mechanisms of apoptosis mediated by lysosomal cathepsins remain to be elucidated in most cases. In order to understand factors involved in the cathepsin B-mediated death pathway, we used the yeast two-hybrid system to hunt for cathepsin B binding proteins. Bikunin and TSRC1 (Gene Bank Accession No. BC027478) were identified and confirmed to interact with cathepsin B in vivo and in vitro. Bikunin is a member of the kunitz-type protease family and acts as an inhibitor against trypsin, plasmin and leukocyte elactase [13], whereas the function of TSRC1 remains unknown. In the presented

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paper, we will report our experimental findings. TNF can induce apoptosis of ovarian cancer cells OV-90 and this process is cathepsin B-depended. Overexpression of bikunin suppresses TNF-triggered apoptosis in OV-90 cells, and TSRC1 overexpression has an opposite effect on apoptosis.

2. Materials and methods

2.1. Chemicals

Recombinant human TNF was kindly provided by Professor Shouyuan Zhao (Fudan University). Transcription inhibitor Actinomycin D (AcD) was purchased from ALEXIS CORPORATION. When cells were treated with TNF, AcD (0.2 μg/ml) supplemented to the medium can inhibit the transcription activation of survival genes and block the activation of NF-κB pathway mediated by TNF. Specific cathepsin B (CTSB) inhibitor CA074Me and its substrate z-RR-AMC were from BIOMOL, pan-caspases inhibitor zVAD-fmk was from CALBIOCHEM. The lysosomotropic fluorochrome acridine orange (AO), propidium iodide (PI) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were from Sigma.

2.2. Plasmid construction and cell transfection

In the procedures of colocalization assay, coding sequence of cathepsin B was in-frame constructed into the fluorescent protein expression vector pDsRed-N1, and bikunin and TSRC1 were cloned into pEGFP-N2 vector, respectively. Coding sequences of bikunin and TSRC1 were cloned into vector pcDNA3.1-myc-HisA(−), respectively, so as to detect effects of overexpression of bikunin or TSRC1 on OV-90 cells apoptosis. Cells were transfected with above plasmids by means of LipofectAMINE (Invitrogen).

2.3. Cell viability assay

10^4 Cells/well were seeded into a 96-well plate and grown overnight. TNF was added at the given concentration and the indicated time point, and cell survival was measured by MTT staining.

2.4. Lysosomal integrity assays

AO-relocation and AO-uptake methods described in detail in Ref. [7] were used to analyze lysosomal integrity. In AO-relocation, cells were first exposed to an AO solution (5 μg /ml) in complete culture medium for 15 min at 37 °C and then rinsed and treated with TNF for 4 h or 8 h. Green cytosolic fluorescence of 10 000 cells per sample was measured by a flow cytometry in the FL1 channel (FACScan; Becton Dickinson). In AO-uptake, cells were first treated with TNF for 8 h and then exposed to AO solution as described above. Red lysosomal fluorescence of 10 000 cells per sample was measured by a flow cytometry in the FL3 channel.

2.5. PI-FACS analysis

Apoptosis was quantified on the basis of the amount of sub-G1-DNA by a flow cytometry as described in detail in Ref. [14].

2.6. Cathepsin B enzymatic assay

Cells were first washed twice with PBS and then lysed. Cathepsin B activity was estimated by adding 10 μl cell lysate and 50 μM z-RR-AMC to cathepsin B reaction buffer (50 mM sodium acetate, 4 mM EDTA, 8 mM DTT, 1 mM pefablock, pH 6.0). The release of 7-amido-4-methylcoumarin (AMC) (excitation 365 nm, emission 440 nm) was measured with a fluorometer (Cary Eclipse Fluorescence Spectrophotometer, VARIAN), and then normalized for the amount of protein [12]. Protein content was determined by Bradford assay (BioRad).

2.7. Yeast two-hybrid screening

Here used methods are described in detail in Ref. [15]. The open reading frame of cathepsin B was fused to Gal4-BD domain on the pGBKT7 vector as bait plasmid and human fetal liver cDNA library was screened. The positive prey plasmids were rescued from yeast cells and then sequenced. A mating test was performed to pluck the specific protein–protein interactions.

2.8. Glutathione S-transferase pull-down assay in vitro

Cathepsin B was cloned into the pGEX-5X-1 vector and expressed in *Escherichia coli* strain BL21. Bikunin was cloned into pGBK7 vector and the Myc-Bikunin fusion protein was generated by the TNT T7 Quick Coupled Transcription/Translation System (Promega). Glutathione *S*-transferase (GST) pull-down assay was performed as described in Ref. [15].

2.9. Coimmunoprecipitation assay in vivo

Coimmunoprecipitation assay was performed as described preciously [15]. Cathepsin B was constructed into vector pCMV-HA, bikunin and TSRC1 were cloned into vector pCMV-Myc, respectively. HeLa cells were cultivated in 6-well plates to 80% confluence and then co-transfected with pCMV-HA-CTSB and pCMV-MYC-Bikunin or pCMV-MYC-TSRC1, respectively. After 48 h of incubation, cells were collected and analyzed so as to detect the interaction between CTSB and bikunin or TSRC1.

3. Results

3.1. Cathepsin B is involved in TNF-induced demise of ovarian cancer cells OV-90

Based on reports, the effects of TNF treatment depend on the cell types. Some cells are sensitive to TNF cytotoxicity after inhibition of NF-κB pathway [2,3]. As shown in Fig. 1A, we screened ovarian cancer cells OV-90, TOV-112 D, prostatic cancer cells PC-3, hepatoma cells Hep3B and SMMC-7721 so as to determine their susceptivity to TNF cytotoxicity. We found that all examined cancer cells were resistant to treatment of TNF alone. However, when the transcription inhibitor actinomycin D was supplemented to the medium, OV-90, PC-3 and SMMC-7721 cells were susceptible to TNF-induced death. For OV-90 cells, its values of MTT assays decreased from 89.0% to 26.7%; for PC-3 cells, from 102.6% to 56.0%; for SMMC-7721 cells, from 91.5% to 26.6%. Either specific cathepsin B inhibitor CA074Me (25 μM) or pan-caspases inhibitor zVAD-fmk (1 μM) could reduce the death of these three TNF-sensitive cell lines (Table 1). Furthermore, the results of apoptosis detected by PI-FACS also showed that 25 μM CA074Me and 1 μM zVAD-fmk suppressed TNF-mediated OV-90 cells apoptosis from 23.9% to 15.9% and 12.6%, respectively (Fig. 1B). Based on above experimental results, it concludes that the cathepsin B and caspases are involved in the OV-90 cells apoptosis induced by TNF.

3.2. TNF induces lysosomal rupture in OV-90 cells

Cathepsin B is experimentally testified to be involved in the TNF-induced apoptosis of OV-90 cells, and this protease is located in lysosomes. It is necessary to study stability of lysosomes during the cell apoptosis for the better understandings of apoptosis mechanisms. The highly sensitive AO-relocation method was used to detect release of AO from ruptured lysosomes to the cytosol. AO is a metachromatic fluorophore that shows strong red fluorescence in lysosomes and weak green fluorescence in cytosol. As shown in Fig. 2A, an enhanced cellular green fluorescence, 113.5% compared with the control, became detectable as early as 4 h after OV-90 cells were treated with TNF/AcD, and then the fluorescent intensity was increased to 139.2% at 8 h. Results of the AO-uptake assay showed an increased numbers of ''pale cells'', i.e., cells with reduced numbers of AO-accumulating lysosomes, after TNF-treating 8 h, which also demonstrated lysosomal rupture (Fig. 2B). Preincubation with either CA074Me or zVAD-fmk

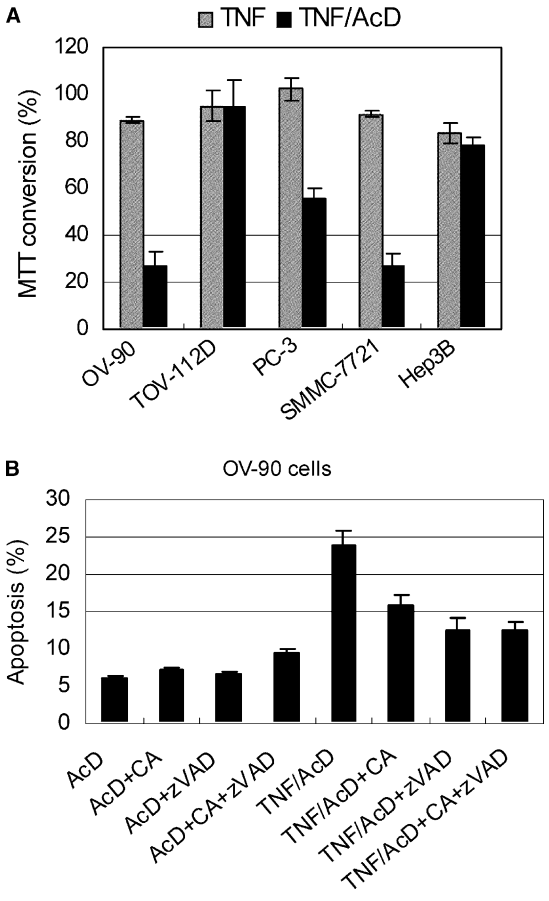

Fig. 1. Sensitivity of human cancer cell lines to TNF treatment and protective effects of cathepsin B and caspases inhibitors on TNF/AcD-induced apoptosis in OV-90 cells. (A) Ovarian cancer cells OV-90 and TOV-112D, prostatic cancer cells PC-3, hepatoma cells Hep3B and SMMC-7721 were screened for their susceptivity to TNF cytotoxicity. The survival cells were measured by MTT assay after 22 h treatment with TNF (1000 U/ml) and AcD (0.2 μg/ml) or TNF (1000 U/ml) alone. (B) OV-90 cells were cotreated 16 h with TNF (1000 U/ml) and AcD (0.2 μg/ml) after preincubation with cathepsin B specific inhibitor CA074Me (25 μM) or pan-caspase inhibitor zVAD-fmk (1 μM) for 2 h and the apoptosis of cells was analyzed by PI-FACS analysis. The values are given as the percentage of apoptotic cells with sub-G1 chromosomal DNA content. All data represent means of at least triplicate determinations ± S.D.

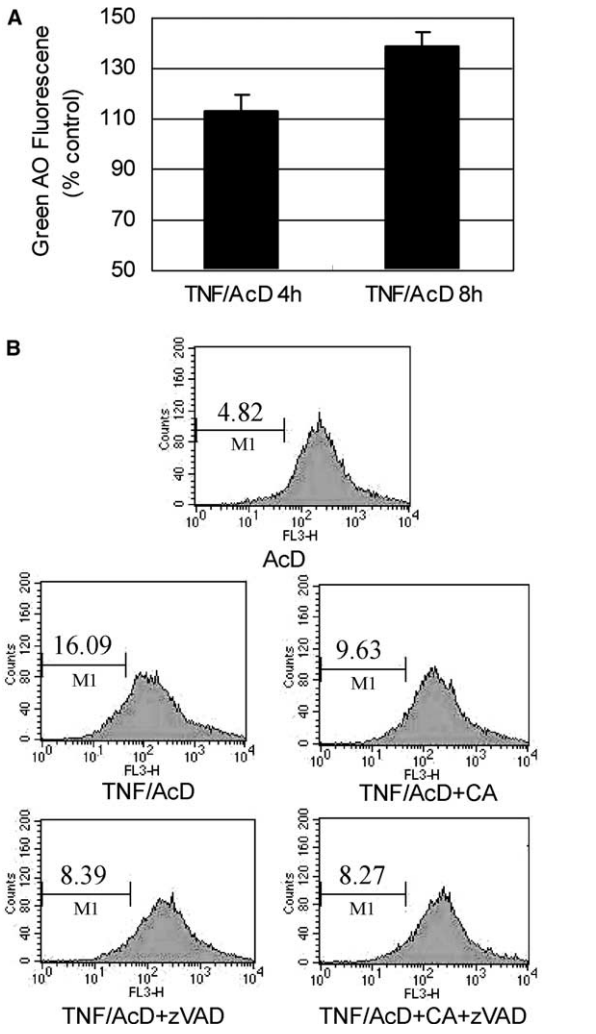

Fig. 2. (A) Early lysosomal rupture assayed with the AO-relocation method. OV-90 cells were stained with AO, rinsed, and cotreated with TNF (1000 U/ml) and AcD (0.2 μg/ml) for 4 h or 8 h. Increase in green-AO fluorescence, indicating release of AO from ruptured lysosomes to the cytosol (10000 cells per sample), was measured by flow cytometry. The green-AO intensity of cells which were not treated with TNF was as control. (B) Lysosomal rupture assayed with the AO-uptake method. After preincubating with or without 25 μM CA074Me (or 1 μM zVAD-fmk) for 2 h, OV-90 cells were treated with TNF/AcD (same concentration as above indicated) for 8 h and then exposed to AO. Intensity of lysosomal fluorescence from 10000 cells per sample was measured. Cells with decreased red fluorescence (''pale'' cells) were gated, and their percentages are indicated. Each data is an average of triplicate samples.

could reduce numbers of ''pale cells'' from 16.09% to 9.63% and 8.39%, respectively. And so it is inferred that the inhibition of cathepsin B or caspases activity would enhance the lysosomal stability.

3.3. Cathepsin B specifically interacts with bikunin or TSRC1

To understanding the pathway of cathepsin B in the cell death, the Clontech Matchmaker Two-Hybrid system 3 and the yeast mating assay were used to screen and identified cathepsin B interacting proteins. As a result, we found that bikunin and TSRC1 interact with cathepsin B. Bikunin belongs

Table 1
Effects of cathepsin B and caspases inhibitors on the death of cancer cells triggered by TNF/AcD

Cell line	MTT conversion (%)				
	TNF	TNF/AcD	TNF/AcD + CA	TNF/AcD + zVAD	TNF/AcD + CA + zVAD
OV-90	89.0 ± 1.6	26.7 ± 6.0	50.1 ± 9.7	67.6 ± 10.3	85.1 ± 4.1
PC-3	102.6 ± 4.8	56.0 ± 4.3	70.6 ± 7.3	71.8 ± 2.8	84.8 ± 3.2
SMMC-7721	91.5 ± 1.2	26.6 ± 5.4	41.4 ± 4.4	48.4 ± 0.7	51.3 ± 2.6

Cells were preincubated with CA074Me (25 μM) or zVAD-fmk (1 μM) for 2 h before a treatment with TNF/AcD as indicated in Fig. 1A. The survival cells were measured by MTT assay. Results represent means of triplicate determinations ± S.D.

to the Kunitz-type protease inhibitor family, while TSRC1 is a novel protein and its function remains unknown. To confirm the interactions between cathepsin B and bikunin or TSRC1 observed in yeast two hybrid, the GST pull-down assay in vitro and coimmunoprecipitation assay in vivo were performed. It was testified that GST-CTSB conjugated on glutathione-Sepharose 4B beads specifically pulled down bikunin while GST alone did not (Fig. 3B). In the procedures of coimmunoprecipitation assay, HeLa cells were cotransfected with pCMV-Myc-CTSB and pCMV-HA-Bikunin or pCMV-HA-TSRC1. Immunoblot analysis verified the efficient expression of CTSB, bikunin and TSRC1 in cell lysates (Fig. 3C). Monoclonal anti-Myc antibody and protein A/G-agarose were added into the cell lysates so as to precipitate Myc-CTSB. HA-Bikunin or HA-TSRC1 was specifically detected in the precipitate by using anti-HA antibody as primary antibody (Fig. 3C). Both the results of in vitro and in vivo binding assays validate that CTSB interacts with bikunin or TSRC1.

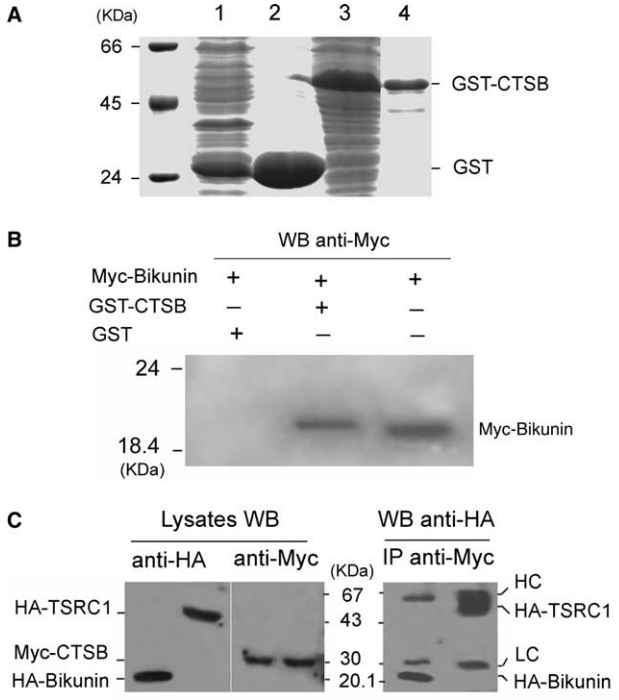

Fig. 3. Validation of interaction of CTSB with bikunin and TSRC1. (A) Expressed and purified GST and GST-CTSB proteins were analyzed by SDS–PAGE. Lanes 1 and 3 represent total soluble proteins of *E. coli* BL21 containing GST and GST-CTSB, respectively; lanes 2 and 4 represent purified GST and GST-CTSB, respectively. (B) The interaction of CTSB with bikunin was analyzed by GST pull-down assay in vitro. GST-CTSB fusion protein immobilized on glutathione-Sepharose 4B beads was incubated with Myc-Bikunin translated in vitro. Binding proteins were immunoblotted with anti-Myc primary antibody. Bikunin binds specifically to GST-CTSB, but not to GST alone. The translation product of bikunin in vitro was used as a positive control. (C) The specific interaction between CTSB and bikunin (or TSRC1) was confirmed by coimmunoprecipitation in vivo. HeLa cells were cotransfected with pCMV-Myc-CTSB and pCMV-HA-bikunin (or pCMV-HA-TSRC1) and the expressions of Myc-CTSB, HA-Bikunin and HA-TSRC1 were analyzed by western blotting with anti-Myc or anti-HA primary antibody, respectively. The lysates from cotransfected cells were immunoprecipitated using mouse anti-Myc antibody. The precipitated proteins were eluted from the protein A/G PLUS agarose and analyzed by western blotting using anti-HA primary antibody. HC and LC represent the heavy chain and light chain of mouse IgG, respectively.

CTSB fused with red fluorescence protein RFP and bikunin or TSRC1 with green fluorescence protein GFP were coexpressed in OV-90 cells. As shown in Fig. 4, Bikunin-GFP or TSRC1-GFP was completely colocalized with CTSB-RFP in cellular lysosomes, displaying spots-like fluorescence as reported in Ref. [16]. These results further support the fact that CTSB interacts with bikunin or TSRC1.

3.4. Overexpression of bikunin or TSRC1 changes the sensitivity of OV-90 cells to TNF-induced cell death

To investigate the functions of CTSB interacting proteins bikunin and TSRC1, bikunin and TSRC1 were cloned into expression vector pcDNA3.1/myc-HisA(−) and transfected OV-90 cells, respectively. After 48 h of transfection, the cells were treated with TNF/AcD and apoptosis of cells was measured. Bikunin and TSRC1 were overexpressed in cells as expected (Fig. 5B). TNF-induced apoptosis of OV-90 cells with the overexpression of bikunin decreased from 31.8% to 19.5%, compared to that of control cells transfected with vain vector; to the contrary, the apoptotic cells with TSRC1 overexpression increased to 59.6%, whereas apoptosis level of OV-90 cells was just 14.4% after preincubation of cathepsin B specific inhibitor CA074Me (Fig. 5A). Based on the experimental data, overexpression of CTSB interacting proteins bikunin or TSRC1 is correlated with TNF-induced OV-90 cells apoptosis. The bikunin overexpression suppresses apoptosis of OV-90 cells triggered by TNF, to the contrary, overexpression of TSRC1 enhances cells apoptosis.

Effects of overexpression of bikunin and TSRC1 on cellular cathepsin B activity are investigated. As shown in Fig. 6, the cellular CTSB activity in OV-90 cells with bikunin overexpression was 58%, compared with that of the control, however, TSRC1 overexpression almost had no influence on cellular CTSB activity. If OV-90 cells were pretreated for 2 h with

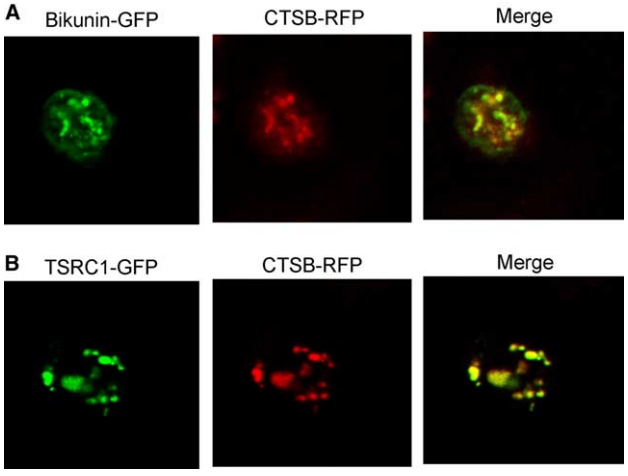

Fig. 4. Colocalization of CTSB with bikunin or TSRC1 in OV-90 cells. (A) OV-90 cells were cotransfected with pDsRed-N1-CTSB and pEGFP-N2-Bikunin. (B) OV-90 cells were cotransfected with pDsRed-N1-CTSB and pEGF-N2-TSRC1. After 48 h of cotransfection, the cells were observed and captured with LEICA TCSNT fluorescent confocal microscope at 480 nm and 568 nm emission wavelengths, respectively. Overlay images demonstrate that CTSB is colocalized with bikunin or TSRC1 in cellular lysosomes.

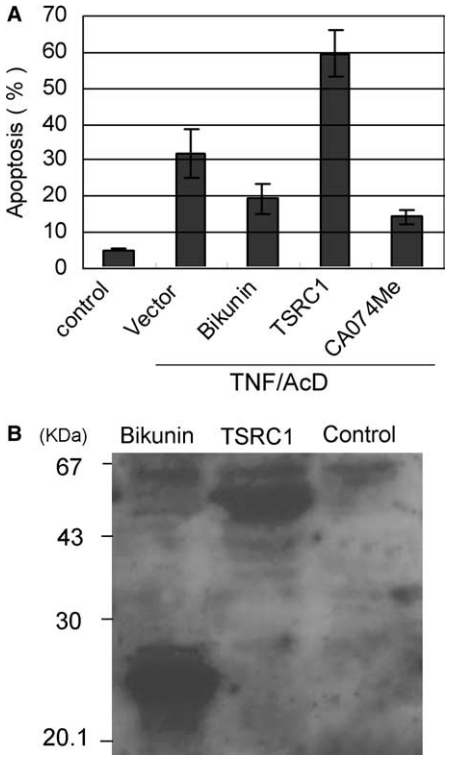

Fig. 5. Influence of bikunin or TSRC1 overexpression in OV-90 cells on TNF-induced apoptosis. Expression vectors containing cDNAs of bikunin or TSRC1 were transfected into OV-90 cells. (A) At 48 h post-transfection, cells were incubated with 1000 U/ml TNF + 0.2 μg/ml AcD, CA074Me (25 μM) was added 2 h before treatment of TNF/AcD. After 16 h of treatment, cells were harvested for PI-FACS analysis. Each data is an average of triplicate samples. (B) Cell extracts were analyzed for the expression of bikunin and TSRC1 by immunoblot.

CA074Me, the cellular CTSB activity was reduced to just 24% of the control's. Accordingly, bikunin might suppress TNF-mediated apoptosis of OV-90 cells by inhibiting enzyme activity of CTSB.

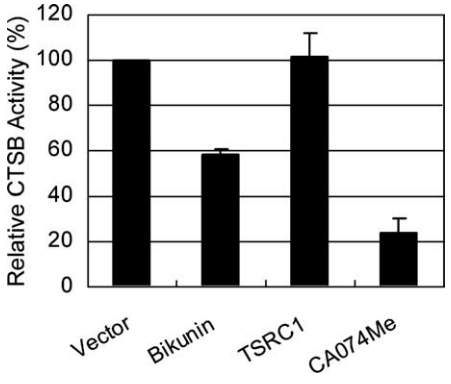

Fig. 6. Effect of bikunin or TSRC1 overexpression on cellular cathepsin B activity in OV-90 cells. After 48 h of transfection as mentioned in Fig. 5, cathepsin B activities of cell extracts were measured. As a control, CA074Me was added in the culture medium 2 h before the harvest of cells. Each data is an average of triplicate samples.

4. Discussion

Based on recent findings, beside the traditional apoptotic process mediated by caspases, the lysosomal cysteine proteases cathepsins, most notably cathepsin B, also participate in apoptotic signaling and they may function upstream or downstream of caspases [6,9–12]. It is well established that many human cancer cells express high level of cathepsins and cytotoxity of TNF causes a variety of cells apoptosis [2,3,17,18]. To this end, we screened several human cancer cell lines for their sensitivity to cytotoxity of TNF. Also, we further investigated the relations between cathepsin B and TNF-triggered cell death. We find that ovarian cancer cells OV-90 is sensitive to the cytotoxity of TNF when the cells were cotreated with transcription inhibitor actinomycin D, and that the apoptosis triggered by TNF/AcD is not only caspase-dependent but also cathepsin B-dependent (Fig. 1). Cathepin B and caspases inhibitors can also protect PC-3 and SMMC-7721 cells from TNF-mediated cell death, indicated in Table 1. And so, our research supports the view that the cellular death responses depend on cell types.

A variety of lysosomal damaging agents or stimulus can result in destability of lysosomes with release of cathepsins to cytosol, leading to apoptosis of cells [6–8]. As shown in Fig. 2, our experiments demonstrate that lysosomes undergo a permeabilization process in TNF-treated OV-90 cells, and that inhibitors against caspases and cathepsin B protect lysosomes from rupture. And so the active caspases and cathepsin B may cause lysosomal destabilization in OV-90 cells treated by TNF. The mechanisms response for TNF-associated lysosomal break down remain unknown in many cases and these questions need to be elucidated at molecular level.

A well accepted fact is that cathepsin B is a major mediator of apoptosis pathway under many circumstances, however, little is known about cellular inhibitors or activators to cathepsin B. We try to find those factors that interact with cathepsin B and that may intervene cathepsin B-mediated death process. Bikunin and TSRC1 are such two proteins interacting with cathepsin B that we have identified by yeast two-hybrid method, and also we have confirmed the interactions by binding assays and colocalization analysis.

TSRC1, an interaction partner of cathepsin B, has 424 amino acids and 5 TSP1 (Thrombospondin type 1 repeats) domains, but its function is unknown. Our study showed that overexpression of TSRC1 enhances OV-90 cells apoptosis induced by TNF, in the meantime, without influence on cellular cathepsin B enzyme activity. It was reported that some TSP1 domain-containing proteins like Thrombospondin 1 and Thrombospondin 2 could induce apoptosis of endothelial cells [19,20], but it is not clear how these proteins work in death pathway. Our results about TSRC1 provide some clues to future investigation in this direction.

Bikunin, another partner of cathepsin B, has a kunitz-type protease inhibitor with inhibitory activity against some proteases such as trypsin, plasmin and granulocyte elactase [13]. Bikunin performs anti-metastatic functions against some cancer cells, but its mechanism remains unclear at present [21]. The bikunin protein is not expressed in normal ovary cells, while ovarian cancer tissues from different patients show wide variability in the expression of bikunin. There is a significant correlation between low expression of bikunin and metastasis of ovarian cancers, and exogenously added bikunin would

reduce invasion and metastasis of ovarian cancer cells [22,23]. For the first time, we verify that, first of all, bikunin interacts with cathepsin B specifically; secondly, overexpression of bikunin protects ovarian cancer cells OV-90 from TNF-induced apoptosis, concomitantly with decrease of cellular cathepsin B activity. The results imply that bikunin may act as an inhibitor against cathepsin B to protect cells from death. Cathepsin B is associated with tumor invasion and metastasis in many experimental and clinical cases, and evaluated as potential diagnostic and prognostic markers for tumors [17,18]. The expression level of cathepsin B is upregulated in many human cancers, such as breast cancer, lung cancer, brain cancer, colorectal cancer, head and neck cancer, and melanoma, and it can be secreted from tumors and cancer cell lines. Cathepsin B works as a cysteine protease to degrade extracellular matrix proteins like collagen IV and lamin. It can activate the precursor form of urokinase-type plasminogen activator (uPA), which is a well-known factor to initiate proteolysis of extracellular matrix, and thus lead to cancer growth and invasion. Studies also show that the expression of cathepsin B is enhanced both in human ovarian cancer tissue and in serum from patients. Furthermore, increase in cathepsin B level corresponds to ovarian cancer development [18]. All available data indicate that cathepsin B plays a critical role in malignant progression. Therefore, it is feasible that bikunin prevents tumor progression partially by inhibiting cathepsin B activity.

Lysosomal cathepsins, particularly cathepsin B, have been testified to be involved in cell death pathways. They cannot only act in concert with caspases, but also independently execute apoptotic function [6–12]. Currently, relations between caspases and cathepsins are poorly understood, and the regulation of cathepsins in cellular apoptosis pathways remains unclear. Cathepsin B has two opposing roles in malignancy, one role is that its proapoptotic features can reduce malignancy; while the other is that secreted cathepsin B protein enhance tumor invasion and metastasis. It is important for successful treatment of cancers to profoundly understand functions of cathepsin B in apoptosis. Our initial results show that cathepsin B and its candidate regulators, bikunin and TSRC1, participate in apoptosis pathway of OV-90 cells. Further deeper research is ongoing.

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